SYNAPTONEMAL COMPLEXES, SERIAL SECTIONS, AND KARYOTYPING

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Introduction

Synaptonemal complexes (SCs) occur between synapsed homologues in meiotic prophase I (for review, Gillies, 1975). In pachynema one SC extends the entire length of each bivalent; hence, the number of SCs corresponds to the haploid chromosome number. Moens and Perkins (1969) first showed that serial section, nuclear reconstruction techniques could be used to count the number of SCs in pachytene nuclei of organisms with small, indistinct chromosomes. Karyotypes based on number and lengths of SCs thus could be determined for organisms with nuclear morphologies that hindered accurate counts and measurements by light microscopy of either somatic or meiotic chromosomes. Karyotype analyses based on serial sections of SCs have been completed for a variety of plants, animals, protists, and fungi. Representative studies of serial sectioned SCs in fungi include those by Gillies (1972), Byers and Goetsch (1975), Zickler (1977), and Tanaka et al (1982). The technique for collecting serial sections outlined in the following paragraphs is essentially that described by Wells (1974), and is used by the present author for karyotyping Plasmodiophoromycetes (e.g., Braselton, 1984).

We should note that improvements are continually being made in whole mount methods for SCs in plants (Albini and Jones, 1984; Gillies, 1981; Stack, 1982) that may be applicable to fungi. When possible, whole mount methods are recommended in addition to serial section methods because (1) whole mount methods are relatively quicker than serial section methods, (2) whole mounts allow for direct measurements of lengths of SCs, and (3) whole mounts eliminate some interpretation difficulties encountered with serial sections when two SCs interlock or end close to each other at the nuclear envelope. There are, however, advantages of serial sections over whole mount methods. These include: (1) there is no danger of losing one or several SCs during preparation in serial section methods; (2) there is no distortion of lengths due to stretching, although there is error introduced by variation of section thickness; (3) three dimensional orientation of SCs and nuclear-associated structures such as centrioles can be determined; (4) cytoplasmic conditions at the time of meiosis can be assayed; and (5) when meiosis does not occur synchronously throughout a population, or occurs within only a few cells of an organism, cells or individuals in the population in pachynema can be located either through examination of flat-embedded specimens prior to mounting and trimming, or through adjacent thick (0.5 μm)-thin section methods.

Support Films

Gray to silver Formvar cast from glass microscope slides may be prepared by standard procedures. If one experiences difficulty with the Formvar separating from the slides, clean the slides with Duraglit (available in the UK) or Wright's Silver Cleaner & Polish (available in the USA); dry slides thoroughly with laboratory tissues after cleaning with silver polish. If one has problems with dirt and (or) holes on the Formvar, commercial preparations of Formvar (e.g., 0.25% Formvar in ethylene dichloride from E.F. Fullam, Inc.) generally provide excellent results. Pick up the Formvar from the water surface with plastic rings (Wells, 1974) or a platform made from Al approx. 0.5-0.75 mm thick. The platform should be approx. 25 x 50 mm and have 20-30, 3/16 inch diam. holes drilled in it (Rowley and Moran, 1975). Carbon coat the Formvar to improve its stability in the electron beam.

Sectioning

Trim blocks so that the smallest face possible is to be cut. Small sections facilitate the location of desired structures when going from one section to the next and also allow for a maximum number of sections per grid. When possible, the present author places 50-80 sections on one grid. Section thickness depends on the type of specimen and the kind of detail desired from the sections. For following SCs in Plasmodiophoromycetes, thick gold (100-130 nm) is preferable. For improved details, however, thinner (silver) sections are recommended. After the ribbons have been cut, arrange them so that the beginning and end of the series are easily recognized. Use freshly-cleaned, 1 x 2 mm copper slot grids without any support film. Eliminate static electricity on the sections and grid with a Zerostat™ antistatic gun. Pick up the sections in a drop of water by slowly lowering the uncoated, static-free grid onto the surface of the water. Transfer the grid with drop of water that contains the sections to carbon-stabilized, Formvar film on support platforms. Place the grid-Formvar-support platform on a slide warmer at approx. 60 C until the water has evaporated and the sections and grid have adhered to the Formvar. Pay particular attention to what side of grid and Formvar the sections are on.

Staining

To remove the grid with sections from the support platform, turn over the platform so that the Formvar-side of the grid is up, and the section-side of the grid is down. Slowly lower the hole with grid over a 5/32 or 3/16 inch diam, vertical, rigidly-held metal rod. Note that the Formvar side of the grid is up, but the specimen is on the side of the Formvar toward the metal rod. Gently remove the grid from the metal rod. Ragged edges of Formvar may be removed by lightly touching the edge of the grid to double sticky tape.

Stain with uranyl acetate and Reynold's lead citrate. Place a drop of stain in a new polystyrene petri dish and float the grid with section-side down on the drop of stain. For rinses, place six individual drops of glass-distilled water in the dish. After the period of staining, transfer the grid to the first water drop for 30 s; continue the rinse sequence by transferring the grid to the remaining drops for 30 s each. Staining times vary depending on thickness of sections, whether or not material was stained en bloc, embedding resin, and type of specimen.

Analysis of Serial Sections of SCs

Follow each SC from section to section and label each profile for future reference (e.g., Figs. 1-15). After all profiles of SCs in the series for a nucleus have been accounted for, trace each SC, section by section, onto an acetate sheet (Fig. 16). If there is a series of cross sections of a given SC that superimpose on each other, be sure to record how many sections there are in the series so that they can be used in the determination of actual length as described later in this paragraph. For each SC, measure

the length of the profile for each section and calculate the estimated length of the SC section by applying the Pythagorean theorem (Gillies, 1972): C = SQR (A^2 + B^2), where C = estimated length, A = measured length of the profile, and B = thickness of the section. Total length of the SC equals the sum of C's. Measurements of the profiles can be made with a wheel-type map reading device, or preferably with a digitizer coupled to a microcomputer. The present author has programs available for distribution that are written in BASIC for an Apple IIe microcomputer with a Houston Instrument Hipad digitizer.

Lengths of central and lateral elements can be determined when they can be followed such as in Ascomycetes (e.g., Gillies, 1972; Zickler, 1977). In cases such as with the Plasmodiophoromycetes, however, the length of the central region is what is measured since central and lateral elements often are not clearly defined. Structures to be scored when present include recombination nodules (Zickler, 1977), centromeres, modifications of lateral elements, and nucleoli. Three- dimensional images can be generated with computer graphics (e.g., Moens and Moens, 1981); or nuclear sizes, positions of centrioles, and sites at the nuclear envelope where SCs terminate can be compared by constructing scale models from dental wax (Fig. 17).

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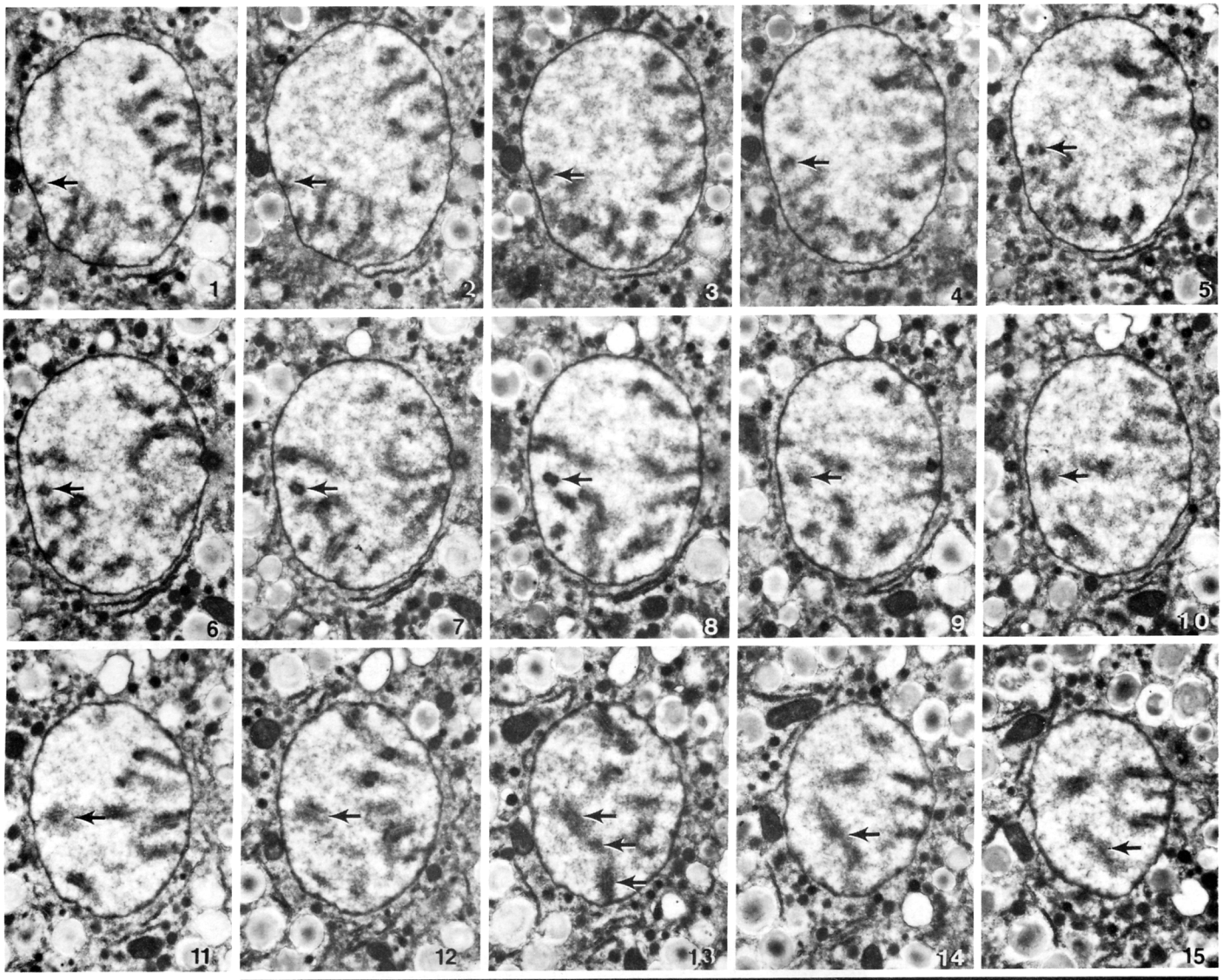
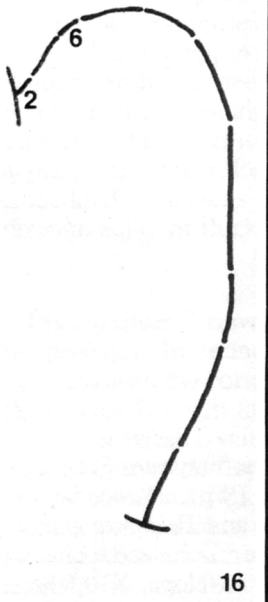
Figures (page over). Figs. 1-15. Serial sections of portion of nucleus of *Polymyxa betae* showing one SC (arrow) completely serial sectioned, X9800. Fig. 16. Composite of tracings of SC in Figs. 1-15, X29, 400. The section thickness for this series was 120 nm, and the total length of the SC was calculated to be 4.29 μm. Fig. 17. Comparison of scale models based on serial sections of pachytene nuclei of the Plasmodiophoromycetes *Plasmodiophora brassicae* (Pb), *Woronina pythii* (Wp), *Plasmodiophora diplantherae* (Pd), *Polymyxa betae* (Pt), and *Polymyxa graminis* (Pg). Black dots represent locations of centrioles and white dots termination sites of SCs at the nuclear envelope, X10,000.





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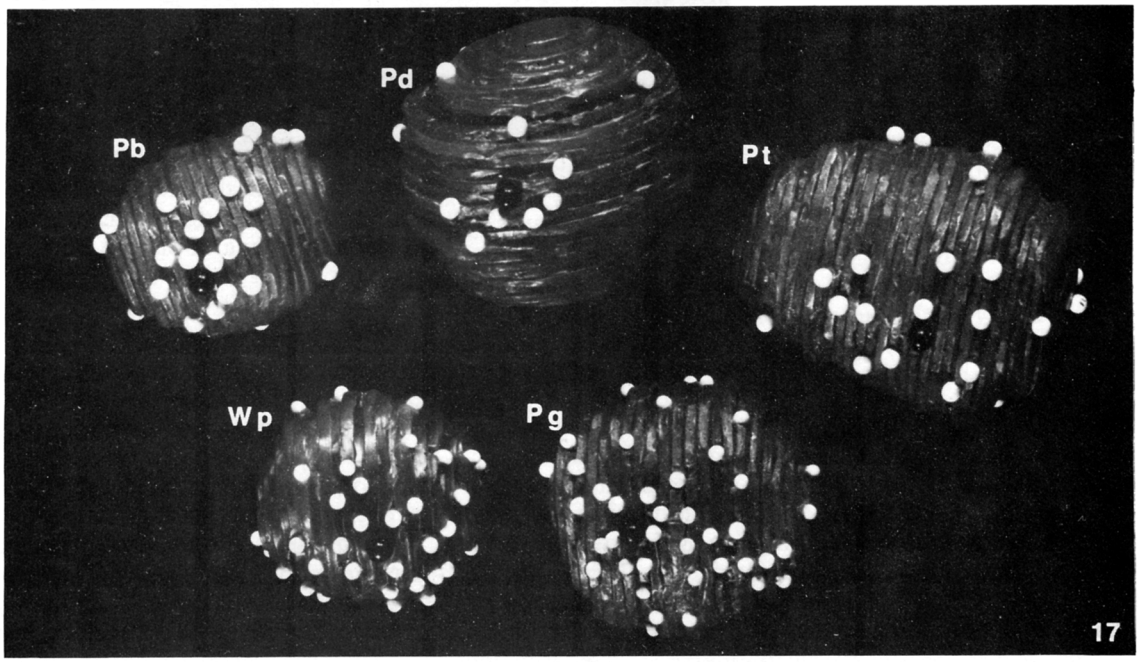

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